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# Rhizobacteria facilitate physiological and biochemical drought tolerance of *Halimodendron halodendron* (Pall.) Voss

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**Abstract:** Growth-promoting bacteria (GPB) have shown promising effects on serving plants against environmental constraints such as drought. Nevertheless, simultaneous effects of different GPB have less been considered for arid land plants and under field conditions. We investigated the effects of single and combined application of GPB, including free-living nitrogen-fixing bacteria (NFB), phosphate solubilizing bacteria (PSB), potassium solubilizing bacteria (KSB), a combination of NFB, PSB, and KSB (NPK), and control, at three drought stress treatments. In order to better understand the interactions between drought and GPB, we measured the morphological, biochemical, and physiological plant traits. The target plant was salt tree (*Halimodendron Halodendron* (Pall.) Voss), a legume shrub native to arid lands of Central and West Asia. All biofertilizer treatments enhanced the growth, physiology, and biochemistry of salt tree seedlings, and there were significant differences among the treatments. KSB and PSB treatments increased photosynthetic pigments, but KSB treatment was more efficient in transpiration rate and stomatal regulation and increased the soluble carbohydrates. PSB treatment had the highest effect on root traits, such as taproot length, root volume, cumulative root length, and the ratio of root to shoot. NFB treatment enhanced root diameter and induced biomass translocation between root systems. However, only the application of mixed biofertilizer (i.e., NPK treatment) was the most significant treatment to improve all plant morphological and physiological characteristics of salt tree under drought stress. Therefore, our results provided improvement of some specific plant traits simultaneous with application of three biofertilizers to increase growth and establishment of salt tree seedlings in the degraded arid lands.

**Keywords:** growth-promoting bacteria; physiological traits; drought stress; biofertilizer; root traits; *Halimodendron Halodendron* (Pall.) Voss

## 1 Introduction

Drought is one of the most critical environmental stresses that limits plant growth, survival, and regeneration in arid environments (Ayangbenro and Babalola, 2021). It affects plant physiological processes and induces specific physiological reactions that assist plant tolerance to restrictive environmental conditions (Mohammadi et al., 2017). Drought causes water loss, reduces leaf

water potential and stomatal conduction, and increases plant transpiration rate (Rodríguez-Gamir et al., 2019). Production and accumulation of osmolytes and antioxidants is a well-known physiological response of plants to drought stress (Khan et al., 2021). Osmolytes, such as proline and soluble carbohydrates, reduce the water potential of plant organs. They generally provide a gradient of potential water surrounding the plant's roots, which enables plants to absorb soil water during the drought (Lombardini and Rossi, 2019). Additionally, the antioxidants, such as phenolic compounds, eliminate active oxygen radicals and maintain and stabilize the cell membrane under drought stress (Hassanein et al., 2021).

Rhizosphere microorganisms, such as growth-promoting bacteria (GPB), have a high potential to modulate the physiological responses of plants in response to water deficiency leading to higher plant survival under stress conditions (Kumar et al., 2020). Plants inoculation with GPB can induce systemic resistance of stressed plants through biochemical, physiological, and morphological processes, such as osmotic regulation and antioxidant defense (Sati et al., 2021). For example, inoculation of *Echinacea purpurea* with *Pseudomonas fluorescens* increased the amount of proline and soluble sugar compared with control and thus improved water use efficiency (Attarzadeh et al., 2019). Inoculation of *Cymbopogon citratus* with *Azotobacter* sp. and *Pseudomonas* sp. increased the antioxidant capacity of plant by increasing the phenol content (Mirzaei et al., 2020). Under severe stress, inoculation of *Mentha piperita* with *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens* increased the non-enzymatic antioxidant proline and total phenol content (Chiappero et al., 2019). Inoculating *Arabidopsis thaliana* with *Azospirillum brasilense* improved plants' performance in drought. *A. brasilense* augmented plant biomass, altered root architecture by increasing lateral roots number, stimulated photosynthetic and photoprotective pigments, and retarded water loss correlated with increased abscisic acid levels. Rhizobacteria inoculation also improved plants' seed yield and survival, thereby reducing stomatal conductance in plants encountered with drought (Ullah et al., 2021). Therefore, the purposive application of microorganisms for the plants growing in the natural ecosystem may also help the growth and establishment of wild plants against natural environmental stresses.

Previous research has mainly focused on the effects of single strains of bacteria and crop plants. In this study, we investigated the impact of multiple and mixed strains of GPB under field conditions to test their ability under the natural environmental conditions. The combination of various strains of GPB can probably increase their effectiveness in the early stages of plant growth. Results of this research can be useful for plant establishment and rangeland restoration in arid areas. We studied the effects of treatments on the salt tree (*Halimodendron haloden*dron (Pall.) Voss), a widespread shrub species in Central and West Asia that naturally grows on dry and saline soil. The results of this research can help restoring dry and alkaline soils in steppe rangelands by using the salt tree shrub.

## 2   Materials and methods

### 2.1   Study area

This experiment was carried out in Garmeh County, North Khorasan Province of Iran (36°58′48″N, 56°17′24″E; 1040 m a.s.l.), from December 2018 to August 2019. The annual average temperature is 16.5°C. Maximum and minimum annual temperatures are 43.1°C and –5.9°C, respectively. The average annual absolute humidity and relative humidity are 46% and 12%, respectively. The long-term average annual rainfall is 158.5 mm, and the potential evapotranspiration is 2535.7 mm. Soil texture is silty clay sand, and soil depth varies from 0 to 40 cm. Soils have low levels of nitrogen (0.45 mg/kg) and phosphorus (8.51 mg/kg), pH is slightly alkaline (7.79), but potassium is about normal (356.67 mg/kg), and electrical conductivity is low (4.32) (Halvin et al., 2013).

### 2.2   Drought treatments

We applied three levels of low, moderate, and high drought intensities. Salt tree seedlings were

irrigated when the water level in the evaporation pan was lost by 30%, 50%, and 70% for the low (LD), moderate (MD), and high (HD) drought treatments, respectively (Ebrahimi et al., 2014). Irrigation levels of 30%, 50%, and 70% were roughly equivalent to 76.0, 127.0, and 178.0 mm of cumulative evaporation from the evaporation pan, respectively. The evaporation pan is a galvanized cylinder with a diameter of 120.7 cm and a depth of 25.0 cm.

## 2.3    Biofertilizer treatments

We applied five biofertilizer treatments, including free-living nitrogen-fixing bacteria (NFB), phosphate solubilizing bacteria (PSB), potassium solubilizing bacteria (KSB), the combination of NFB, PSB, and KSB (NPK), and control. The bacterial population density was estimated as $1.0 \times 10^7$ cells per milliliter of inoculum. PSB was composed of *Bacillus* sp. and *Pseudomonas* sp., KSB was a mixture of *Thiobacillus* sp., and NFB composed of *Azotobacter* sp., *Azospirillum* sp., and *Bacillus* sp.. All strains were collected from the natural ecosystems and croplands of Northeast Iran. Total concentration was similar in all treatments, i.e., 0.500 L of single treatments (NFB, PSB, and KSB) per 100.000 L of irrigation water. For the mixed treatment (NPK), we applied 0.167 L for each biofertilizer to make sure that total concentration is 0.5%.

## 2.4    Seed and seedling cultivation

At the beginning of December 2018, seeds of salt tree were placed in the bacterial fertilizer solution (0.5% concentration) for 15 min (Ullah et al., 2017). The inoculated seeds were dried in the shade and immediately planted in pots. Biofertilizers were added into pots via the irrigation water three times, i.e., once every two months. After six months of growth in the pots, salt tree seedlings were transplanted into the field in early June 2019. They were irrigated with water enriched by biofertilizers at the time of seedling transplantations. Drought treatments were started two weeks after seedling establishment in the field and lasted up to two months.

## 2.5    Experimental design and data analysis

The experiment was performed as split plots in a randomized complete block design with three replications (blocks). We applied three drought treatments (LD, MD, and HD) as the main plot, five biofertilizer treatments (NFB, PSB, KSB, NPK, and control) as subplots. Each experimental unit was a 1 m×1 m quadrat that received a certain level of the combined treatment of drought and biofertilizer, and was replicated three times. Therefore, the experiment contained 135 experimental units. Data were analyzed by performing general linear regression models using Minitab18 software. Then, the means were compared by least significant difference (LSD) test at a 5% probability level.

## 2.6    Measuring plant traits

The youngest fully expanded leaves were sampled to determine the morphological and physiological responses to fertilizer and drought treatments. The measurements were conducted one week after the end of drought treatment and before the flowering stage of salt tree seedlings.

## 2.7    Physiological traits

Net photosynthesis, transpiration, stomatal conductance, and substomatal $CO_2$ concentration were measured by using an infrared gas analyzers (Model LCA4, ADC BioScientific Ltd., Herts, UK). Leaf chlorophyll fluorescence was measured by fluorometer (Model OS1 FL, Opti-Sciences Inc., Hudson, USA) under light conditions. All photosynthetic and chlorophyll fluorescence measurements were performed from 8:00 to 10:00 (LST) on sunny days. In addition, we calculated the Photosystem II quantum yield (PSΠΦ) based on the method conducted by Maxwell and Johnson (2000). To measure the concentration of photosynthetic pigments, we isolated 100-mg fresh leaves from fully expanded young leaves. Pigments were extracted using 96% ethanol (Şükran et al., 1998). The absorption was measured at 470, 653, and 666 nm using a spectrophotometer (Jenway™ 6305 UV/Visible Spectrophotometer Fisher Scientific, Leicestershire, Cambridge, UK). In order to measure the abaxial and adaxial stomatal density of

the plant leaf, we used top coat nail polish, and calculated the number of pores by an optical microscope. Their density was calculated in square centimeters. The leaf area was measured with a leaf area meter (WinDIAS Leaf Image Analysis System, Delta-T Devices Ltd., Cambridge, UK).

## 2.8 Biochemical traits

We determined leaf soluble carbohydrates based on phenol, sulfuric acid, and glucose standards (Dubois et al., 1956). Then, we measured the total phenol concentration in fresh leaf samples and the proline concentration according to the Folin-Ciocalteu method (Singleton and Rossi, 1965) and the method conducted by Bates et al. (1998), respectively. Measurement of free radical scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) was performed using the method of Abe et al. (1998). We also determined the osmotic potential of the leaf using an osmometer (Model OM 802-D, Vogel GmbH & Co. KG., Giessen, Germany) based on the freezing point method. Finally, we calculated the amount of osmolality in the leaf based on the combination of van't Hoff's law and the amount of leaf water.

## 2.9 Morphological characteristics

At the end of drought stress, plant height and taproot length were measured with a ruler. After 48 h in the oven at 70°C, dry weight of roots and shoots were measured by a digital scale. The volume of roots was calculated by the method of volume difference created after placing the roots in a specific volume of water (Archimedes Law). Root properties were measured by a scanner (CB50EJ Root Analyzer System, Delta-T Devices Ltd., Cambridge, UK) and its computer software.

## 3 Results

### 3.1 Morphological plant traits

Effects of biofertilizer treatments and drought levels on biomass and morphological traits of salt tree were compared (Table 1). There were some general trends, i.e., plant biomass and morphological traits decreased with the increase of drought levels, but increased with the application of biofertilizers. However, the effects of biofertilizer treatments varied depending on drought levels and morphological traits.

The total plant height varied significantly among all biofertilizer treatments and all drought levels. At LD level, the plant height under NPK treatment was the highest, while at HD level, the plant height under control treatment was the lowest. Biofertilizer treatments led to the increase of plant height for salt tree seedlings, and this effect was consistent at all drought levels. However, higher plant height was found under NPK and NFB treatments, while PSB and KSB treatments were in the next order. The same results were also found for the stem dry weight (Table 1).

Biofertilizer treatments also increased the leaf dry weight, but the greatest value was found under NPK treatment. The effects of single application of biofertilizers (NFB, PSB, and KSB) were diminished under drought conditions. At HD level, only NPK treatment led to significantly higher leaf dry weight than the control treatment (Table 1). Application of all biofertilizers significantly increased leaf area. The largest leaf area was found under NPK treatment. However, the effects of biofertilizers were reduced by increasing drought levels. There were no differences in leaf area among all biofertilizer treatments at HD level (Table 1).

At HD level, the taproot length under PSB treatment was significantly higher than that under other treatments. With increasing drought levels, root volume was unchanged under PSB treatment but reduced under all other biofertilizer treatments. The highest cumulative root length was observed under PSB treatment at LD level. The highest root dry weight was observed at LD level under NPK treatment. Nevertheless, with the increase of drought levels, the greatest decrease in root dry weight was observed under KSB treatment. The mean root diameter showed an enhanced trend under biofertilizer treatments, with the highest being found under NFB treatment at HD level. The ratio of root to shoot decreased under KSB treatment, but increased under other biofertilizer treatments (Table 1).

**Table 1**　Effect of different biofertilizer treatments and drought levels on biomass and morphological traits of salt tree

| Biomass and morphological trait | Drought level | Biofertilizer treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | NFB | PSB | KSB | NPK |
| Total plant height (cm) | LD | 19.72±1.21[h] | 31.56±1.33[c] | 23.83±1.83[f] | 22.44±1.79[g] | 41.36±2.10[a] |
| | MD | 14.11±0.87[j] | 26.83±0.63[e] | 17.67±1.04[i] | 17.12±0.86[i] | 34.72±1.04[b] |
| | HD | 7.61±1.38[l] | 21.67±1.33[g] | 11.61±1.35[k] | 13.33±1.49[j] | 29.61±0.87[d] |
| Stem dry weight (g/Plant) | LD | 0.670±0.028[defgh] | 0.924±0.175[d] | 0.870±0.140[de] | 1.250±0.111[c] | 2.140±0.159[a] |
| | MD | 0.505±0.137[ghi] | 0.798±0.007[defg] | 0.644±0.026[defgh] | 0.589±0.112[efghi] | 1.560±0.152[b] |
| | HD | 0.312±0.085[i] | 0.582±0.045[efghi] | 0.416±0.061[hi] | 0.540±0.127[fghi] | 0.825±0.088[def] |
| Leaf dry weight (g/Plant) | LD | 0.837±0.117[de] | 0.995±0.101[cd] | 1.450±0.110[b] | 1.580±0.091[b] | 2.180±0.212[a] |
| | MD | 0.383±0.081[g] | 0.576±0.060[fg] | 0.654±0.063[ef] | 0.572±0.060[fg] | 1.190±0.112[c] |
| | HD | 0.364±0.020[g] | 0.460±0.084[fg] | 0.458±0.098[fg] | 0.474±0.110[fg] | 0.630±0.046[ef] |
| Leaf area (cm²/Plant) | LD | 57.1±14.4[cd] | 74.8±5.6[bc] | 90.3±9.2[b] | 119.7±23.0[a] | 131.0±15.0[a] |
| | MD | 27.4±6.1[e] | 46.2±7.5[de] | 56.4±8.4[cd] | 46.7±5.7[de] | 80.4±14.8[bc] |
| | HD | 24.6±1.4[e] | 34.8±5.8[de] | 33.5±6.5[de] | 33.7±6.9[de] | 35.4±5.4[de] |
| Taproot length (cm) | LD | 37.9±2.0[cde] | 33.0±0.7[def] | 45.4±2.0[a] | 43.7±2.6[ab] | 44.5±3.4[ab] |
| | MD | 30.2±0.6[f] | 37.5±4.2[cde] | 32.5±0.3[ef] | 30.9±3.2[f] | 45.6±3.4[a] |
| | HD | 37.0±4.7[cde] | 39.6±5.2[bc] | 46.0±7.0[a] | 36.6±4.8[cde] | 37.9±7.0[cd] |
| Root dry weight (g/plant) | LD | 0.678±0.101[ef] | 0.749±0.130[def] | 1.210±0.101[bc] | 2.160±0.364[a] | 2.180±0.225[a] |
| | MD | 0.689±0.019[ef] | 1.110±0.151[cd] | 0.900±0.017[cde] | 0.730±0.021[def] | 1.580±0.198[b] |
| | HD | 0.964±0.055[cde] | 0.912±0.253[cde] | 0.907±0.142[cde] | 0.364±0.016[f] | 0.680±0.051[ef] |
| Root volume (cm³/plant) | LD | 2.00±0.01[fgh] | 3.00±0.01[def] | 3.67±0.88[cd] | 5.18±0.82[ab] | 6.33±0.67[a] |
| | MD | 2.00±0.00[fgh] | 3.67±0.33[cd] | 3.63±0.67[cd] | 1.33±0.33[gh] | 4.33±0.33[bc] |
| | HD | 2.00±0.58[fgh] | 2.42±0.42[efg] | 3.33±0.33[cde] | 1.00±0.01[h] | 2.33±0.33[efg] |
| Cumulative root length (m) | LD | 52.5±18.6[cd] | 41.4±8.3[cde] | 142.0±12.8[a] | 56.4±17.9[c] | 95.3±10.7[b] |
| | MD | 42.6±11.7[cde] | 32.5±4.6[cde] | 30.1±5.5[cde] | 95.0±14.2[b] | 58.5±13.2[c] |
| | HD | 21.2±4.7[de] | 16.9±0.7[e] | 47.3±18.6[cde] | 26.1±6.9[cde] | 26.0±4.4[cde] |
| Mean root diameter (mm) | LD | 0.797±0.086[abc] | 0.776±0.087[bcd] | 0.402±0.149[def] | 0.956±0.081[ab] | 0.504±0.120[cdef] |
| | MD | 0.331±0.060[f] | 0.783±0.133[abcd] | 0.856±0.216[abc] | 0.376±0.102[ef] | 0.730±0.244[bcde] |
| | HD | 1.046±0.125[ab] | 1.170±0.112[a] | 0.669±0.085[bcdef] | 0.700±0.157[bcdef] | 0.696±0.083[bcdef] |
| Ratio of root to shoot | LD | 0.527±0.066[cdef] | 0.387±0.055[f] | 0.461±0.096[ef] | 0.781±0.163[bcde] | 0.504±0.046[def] |
| | MD | 0.700±0.055[bcdef] | 0.814±0.129[bcde] | 0.888±0.232[bc] | 0.658±0.105[cdef] | 0.582±0.083[cdef] |
| | HD | 1.036±0.155[b] | 0.849±0.146[bcd] | 1.506±0.264[a] | 0.362±0.030[f] | 0.469±0.017[ef] |

Note: Control, without the application of biofertilizer; NFB, free-living nitrogen-fixing bacteria; PSB, phosphate solubilizing bacteria; KSB, potassium solubilizing bacteria; NPK, the combination of NFB, PSB, and KSB; LD, low drought; MD, moderate drought; HD, high drought. Different lowercase letters indicate significant differences among different drought levels and biofertilizer treatments within the same trait ($P \leq 0.05$). Mean±SE.

### 3.2　Photosynthetic traits

The drought levels significantly affected the photosynthetic traits of salt tree seedlings under all biofertilizer treatments (Table 2). The drought effects varied along the drought gradient, among biofertilizer treatments, and depending on the measured parameters.

The highest photosynthesis rate was found under NPK treatment. A single application of biofertilizer treatments (NFB, PSB, and KSB) similarly increased the photosynthesis rate of salt tree, compared to control treatment. However, either of them showed no significant differences (Table 2).

Biofertilizer treatments performed differently in terms of the transpiration rate under drought levels. While salt tree seedlings showed a similar transpiration rate at LD level, the difference among biofertilizer treatments became more evident as drought got more intense at MD and HD levels. NPK and KSB treatments helped salt tree seedlings to regulate stomatal conductance to lower levels at MD and HD levels (Table 2).

All biofertilizer treatments increased substomatal $CO_2$ concentration by increasing drought stress. However, the only significant increase was under NPK treatment. Adaxial and abaxial stomatal density were gradually increased by increasing the drought levels. However, the changes were inconsistent and varied among the treatments. At all drought levels, the highest adaxial and abaxial stomatal density were found under control, and the lowest values were under NPK treatment (Table 2).

**Table 2**  Effect of different biofertilizer treatments and drought levels on photosynthetic traits of salt tree

| Photosynthetic trait | Drought level | Biofertilizer treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | NFB | PSB | KSB | NPK |
| Photosynthetic rate ($\mu mol/(m^2 \cdot s)$) | LD | 18.3±1.5[cd] | 27.9±3.6[bc] | 27.5±3.1[bc] | 26.3±3.2[bc] | 58.1±3.1[a] |
| | MD | 13.7±3.6[d] | 27.6±5.0[bc] | 25.8±2.9[bc] | 21.6±4.2[cd] | 35.5±5.1[b] |
| | HD | 10.7±2.3[d] | 19.7±6.1[cd] | 21.3±3.7[cd] | 20.8±4.6[cd] | 27.1±4.0[bc] |
| Transpiration rate ($mmol/(m^2 \cdot s)$) | LD | 0.197±0.012[g] | 0.373±0.121[g] | 0.397±0.154[g] | 0.590±0.208[efg] | 0.433±0.101[g] |
| | MD | 0.525±0.102[fg] | 1.120±0.220[cde] | 0.640±0.139[defg] | 1.050±0.102[cdef] | 1.180±0.285[cd] |
| | HD | 1.310±0.772[c] | 2.590±0.406[a] | 1.900±0.617[b] | 0.993±0.384[cdef] | 0.542±0.312[fg] |
| Stomatal conductance ($mmol/(m^2 \cdot s)$) | LD | 0.053±0.003[e] | 0.113±0.053[cde] | 0.097±0.048[cde] | 0.173±0.085[cde] | 0.087±0.026[cde] |
| | MD | 0.110±0.055[cde] | 0.270±0.069[b] | 0.110±0.028[cde] | 0.299±0.040[b] | 0.228±0.053[bc] |
| | HD | 0.212±0.167[bcd] | 0.680±0.152[a] | 0.283±0.141[b] | 0.207±0.098[bcd] | 0.063±0.049[d] |
| Stomatal resistance (m/s) | LD | 3273±382[def] | 5382±909[abc] | 3029±1055[def] | 2534±862[f] | 2919±416[ef] |
| | MD | 3722±162[cdef] | 3150±821[def] | 4337±696[cde] | 4682±241[bcd] | 6052±358[ab] |
| | HD | 2737±48[ef] | 4183±725[cdef] | 3686±487[def] | 3344±178[def] | 6585±832[a] |
| Substomatal $CO_2$ concentration ($mg/m^3$) | LD | 292±24[bc] | 303±19[b] | 322±4[ab] | 355±16[ab] | 296±40[b] |
| | MD | 348±20[ab] | 352±17[ab] | 395±34[a] | 353±21[ab] | 356±17[ab] |
| | HD | 356±25[ab] | 360±9[ab] | 325±37[ab] | 306±23[b] | 218±38[c] |
| Adaxial stomatal density (number/cm²) | LD | 409±16[a] | 380±28[ab] | 413±7[a] | 257±41[e] | 291±8[cde] |
| | MD | 282±33[de] | 352±5[abc] | 352±29[abc] | 329±27[bcd] | 280±26[de] |
| | HD | 352±26[abc] | 356±10[abc] | 270±32[de] | 275±25[de] | 297±4[cde] |
| Abaxial stomatal density (number/cm²) | LD | 350±16[b] | 290±0[bcde] | 288±19[cde] | 261±15[cdef] | 253±29[cdef] |
| | MD | 432±19[a] | 236±19[ef] | 299±29[bcd] | 252±26[cdef] | 222±27[f] |
| | HD | 310±20[bc] | 292±10[bcde] | 275±22[cdef] | 248±28[def] | 241±27[def] |

Note: Different lowercase letters indicate significant differences among different drought levels and biofertilizer treatments within the same photosynthetic trait ($P \leq 0.05$). Mean±SE.

The minimum chlorophyll fluorescence of the leaves adapted to light showed a decreased trend by increasing drought levels, the only exception was under control treatment where it was increased. However, the minimum chlorophyll fluorescence showed the highest reduction under KSB treatment (Fig. 1). The maximum chlorophyll fluorescence of light-adapted leaves decreased under NFB and KSB treatments but increased under other treatments, with increasing drought levels. The highest decrease was observed under NFB treatment, and the highest increase was observed under control treatment. The quantum efficiency of photosystem II showed an increased trend with increasing drought levels. This trait increased under all biofertilizer treatments. The highest increase was found under NPK treatment, and the lowest increase was observed under control treatment (Fig. 1).
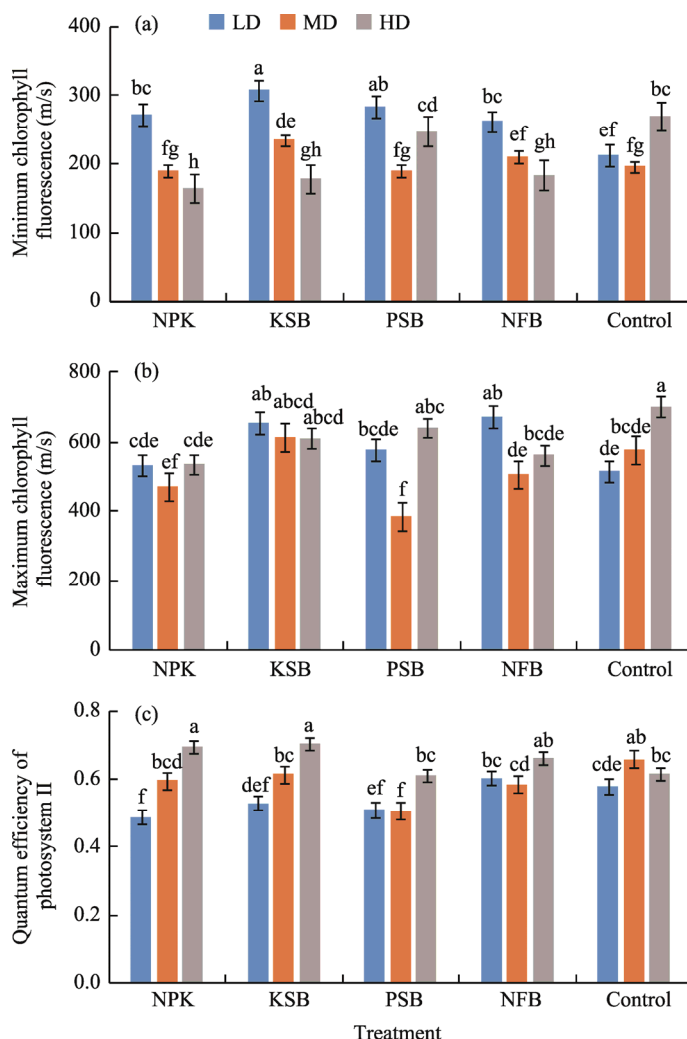
**Fig. 1** Effects of different drought levels and biofertilizer treatments on leaf chlorophyll fluorescence of salt tree. (a), minimum chlorophyll fluorescence; (b), maximum chlorophyll fluorescence; (c), quantum efficiency of photosystem II. Control, without the application of biofertilizer; NFB, free-living nitrogen-fixing bacteria; PSB, phosphate solubilizing bacteria; KSB, potassium solubilizing bacteria; NPK, the combination of NFB, PSB, and KSB; LD, low drought; MD, moderate drought; HD, high drought. Different lowercase letters indicate significantly difference among drought levels and biofertilizer treatments ($P \leq 0.05$). Bars are stand errors.

The concentrations of all photosynthesis pigments, i.e., chlorophyll a, chlorophyll b, carotenoids, and total pigments, showed a decreased trend with increasing drought levels (Fig. 2). At all drought levels, the highest concentration of photosynthesis pigments was found under NPK treatment. A single application of biofertilizer also significantly increased the photosynthesis pigment, but their effects followed the order of KSB=PSB≥NFB.

### 3.3 Biochemical traits

The drought levels significantly affected the biochemical traits of salt tree seedlings. However, its effect varied along the drought gradient, among biofertilizer treatments, and depending on the measured parameters. In general, KSB and NPK treatments were more effective on promoting the salt tree's biochemical traits at different drought levels (Table 3).

The amount of soluble carbohydrate was significantly higher under KSB treatment than other treatments, whereas other biofertilizer treatments significantly decreased it as compared with the control treatment. The highest differences among biofertilizer treatments were found at HD level (Table 3).
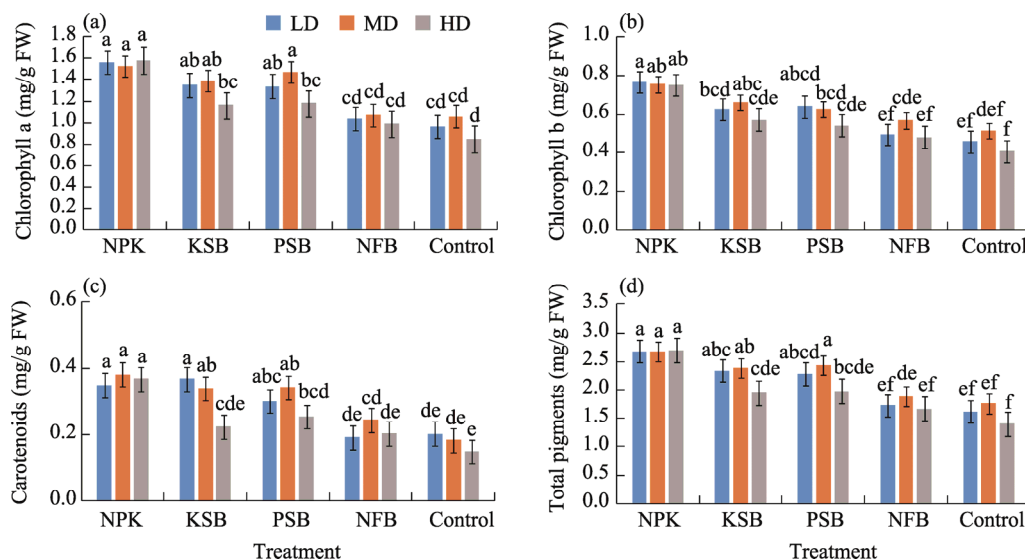
**Fig. 2** Effect of different drought levels and biofertilizer treatments on the concentration of photosynthetic pigments of salt tree. (a), chlorophyll a; (b), chlorophyll b; (c), carotenoids; (d), total pigments. Different lowercase letters indicate significantly difference among drought levels and biofertilizer treatments ($P \leq 0.05$). Bars are stand errors.

**Table 3** Effect of different drought levels and biofertilizer treatments on biochemical traits of salt tree

| Biochemical trait | Drought level | Biofertilizer treatment | | | | |
|---|---|---|---|---|---|---|
| | | Control | NFB | PSB | KSB | NPK |
| Soluble carbohydrates (mg/g) | LD | 1.48±0.06[b] | 1.13±0.08[cd] | 1.53±0.05[b] | 1.85±0.09[a] | 1.08±0.03[cd] |
| | MD | 1.48±0.19[b] | 1.29±0.09[bc] | 1.07±0.03[cd] | 1.33±0.21[bc] | 1.20±0.09[cd] |
| | HD | 1.02±0.03[d] | 1.17 ±0.13[cd] | 1.09±0.09[cd] | 1.97±0.13[a] | 0.95±0.04[d] |
| Proline (mg/g) | LD | 0.010±0.004[d] | 0.419±0.129[cd] | 0.347±0.158[cd] | 0.201±0.187[cd] | 0.009±0.004[d] |
| | MD | 0.210±0.155[cd] | 1.280±0.251[b] | 1.710±0.384[ab] | 0.615±0.274[c] | 0.656±0.009[de] |
| | HD | 0.483±0.092[cd] | 0.217±0.162[cd] | 0.096±0.051[d] | 0.096±0.055[d] | 1.820±0.171[a] |
| Phenol (mg/g) | LD | 3.60±0.14[bc] | 2.42±0.23[de] | 2.03±0.36[e] | 3.43±0.58[bc] | 4.72±0.22[a] |
| | MD | 4.00±0.42[ab] | 3.54±0.22[bc] | 3.06±0.24[cd] | 3.30±0.32[bc] | 3.29±0.44[bc] |
| | HD | 3.53±0.43[bc] | 3.50±0.29[bc] | 3.69±0.18[bc] | 3.28±0.42[bc] | 2.95±0.12[cd] |
| DPPH (mg/g) | LD | 0.162±0.030[efg] | 0.155±0.006[efg] | 0.140±0.014[fg] | 0.181±0.027[defg] | 0.209±0.035[bcde] |
| | MD | 0.215±0.066[bcde] | 0.259±0.052[abc] | 0.168±0.035[efg] | 0.268±0.052[ab] | 0.183±0.043[defg] |
| | HD | 0.237±0.048[abcd] | 0.132±0.012[g] | 0.179±0.039[defg] | 0.201±0.020[cdef] | 0.283±0.044[a] |
| Osmotic potential (MPa) | LD | 3.18±0.19[d] | 5.92±0.20[bc] | 4.64±0.75[cd] | 3.43±1.02[d] | 4.39±1.28[cd] |
| | MD | 4.75±0.46[bcd] | 4.68±0.50[cd] | 3.73±0.02[d] | 3.64±0.30[d] | 5.01±0.48[bcd] |
| | HD | 4.31±0.96[cd] | 4.91±0.39[bcd] | 6.56±0.94[ab] | 5.78±0.46[bc] | 8.05±0.62[a] |

Note: DPPH, 2,2-diphenyl-1-picrylhydrazyl. Different lowercase letters indicate significant differences among different drought levels and biofertilizer treatments within the same biochemical trait ($P \leq 0.05$). Mean±SE.

The amount of proline showed an increased trend along the drought levels. The highest amount of proline was observed under NPK treatment at HD level. Although a single application of biofertilizer led to increased proline at MD level, their effect was diminished at HD level. Total phenol content was the highest under NPK treatment at LD level. However, the difference among biofertilizer treatments was reduced at MD and HD levels (Table 3). We did not find a clear trend for changes in the inhibition degree of 2,2-diphenyl-1-picrylhydrazyl (DPPH) at different drought levels. The only variation was an increase of DPPH under increasing drought levels from LD to

HD. DPPH was constantly and significantly higher under NPK treatment than a single application of biofertilizer (NFB, KSP, and PSB) at all drought levels (Table 3). All biofertilizer treatments could reduce the plant's osmotic potential at HD level. However, the highest difference was found between NPK treatment and control treatment at HD level (Table 3).

Pearson correlation coefficient for the measured traits were given in Table 4. There was a high positive correlation among root traits. The only negative correlation in root traits was observed between cumulative root length and mean root diameter ($r= -0.612$; Table 4).

**Table 4** Correlation coefficients between root traits and biochemical traits of salt tree

| | Taproot length | Root dry weight | Root volume | Cumulative root length | Mean root diameter | Ratio of root to shoot | Soluble carbohy-drates | Proline | Phenol | DPPH | Osmotic potential |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Taproot length | 1.000 | | | | | | | | | | |
| Root dry weight | 0.546** | 1.000 | | | | | | | | | |
| Root volume | 0.476** | 0.867** | 1.000 | | | | | | | | |
| Cumulative root length | 0.306* | 0.399** | 0.341* | 1.000 | | | | | | | |
| Mean root diameter | 0.168 | 0.097 | 0.028 | −0.612** | 1.000 | | | | | | |
| Ratio of root to shoot | 0.131 | 0.088 | 0.146 | −0.271 | 0.320* | 1.000 | | | | | |
| Soluble carbohydrates | 0.027 | 0.038 | −0.124 | 0.187 | −0.088 | −0.214 | 1.000 | | | | |
| Proline | −0.173 | −0.145 | −0.062 | −0.220 | 0.045 | −0.052 | −0.414** | 1.000 | | | |
| Phenol | 0.216 | 0.335* | 0.288 | −0.090 | −0.021 | 0.199 | −0.050 | −0.183 | 1.000 | | |
| DPPH | 0.051 | −0.101 | −0.172 | −0.130 | −0.142 | −0.074 | −0.252 | 0.282 | 0.201 | 1.000 | |
| Osmotic potential | 0.008 | −0.339* | −0.215 | −0.324* | 0.006 | −0.001 | −0.294* | 0.208 | −0.127 | 0.148 | 1.000 |

Note: * and ** represent significant at 5% and 1% levels, respectively.

## 4 Discussion

### 4.1 Morphological responses of salt tree to drought and biofertilizer

Growth-promoting rhizobacteria alter the distribution patterns of assimilates in plants and affect growth patterns (Khan et al., 2019a). Under drought stress, plants usually reduce leaf area and growth rate to decrease transpiration rate and increase water use efficiency (Kumar et al., 2017). However, in this research, the drought treatment led to a minor negative effect on height of salt tree under NPK treatment.

PSB treatment had the most significant positive effects on taproot length, root volume, cumulative root length, and the ratio of root to shoot at different drought levels. PSB, by providing phosphorous and producing phytohormones and enzymes, may reduce ethylene levels in the roots, resulting in an increased root length, lateral root density, and subsequently, water absorption is facilitated (Dey et al., 2021; Yuan et al., 2022)

The availability of potassium by KSB treatment has improved the photosynthetic traits of the leaves. Thus, the transfer of photosynthetic assimilates to the roots increased the root dry weight at LD level. Under stress conditions, the plant devotes most of its photosynthetic production to the accumulation of dry matter in the root system to increase the absorption capacity of roots (Glanz-Idan and Wolf, 2020). However, at HD level, root dry weight decreased sharply, and the ratio of root to shoot decreased. The plant seems to have transferred a large amount of its dry matter to the leaves and shoots to improve its photosynthesis rate and survival. Reports indicated that the plant changes the distribution patterns of photosynthesis and dry matter under the influence of stress or GPB (Liu et al., 2016; Yaghoubi Khanghahi et al., 2019).

NFB treatment increased the plant diameter significantly under drought stress. Because lateral

root maintenance is costly for the plant (Ryan et al., 1996), plants reduce their lateral roots and increase their axial root length to increase survival and water uptake from the soil, in response to drought stress (Kim et al., 2020). Increasing the total root length is associated with a decrease in root diameter, indicating that stressed plants can trade off carbon in their root system (Awad et al., 2018). The negative correlation between cumulative root length and mean root diameter confirms this statement ($r= -0.612$; Table 4). Changing the diameter is one of the ways that plant roots can respond to a changing environment (Lozano et al., 2020). The penetration of roots into the hard layers is achieved by having a large root diameter that resists bending (Vanhees et al., 2022), and nitrogen availability improve the root diameter in these conditions (Moretti et al., 2020). Salt tree increased the length or diameter of its axial root for its survival and water absorption (Lynch, 2018) that has been reported in various reports (Ju et al., 2018; Li et al., 2020).

## 4.2    Photosynthetic responses of salt tree to drought and biofertilizer

In this study, photosynthesis rate was higher under all biofertilizer treatments than under control treatment. However, the effects of biofertilizer varied among the treatments and at various drought levels. In response to drought, the less photosynthetic reduction was found under KSB treatment than under NFB and PSB treatments. Among plant nutrients, potassium plays a vital role in photosynthetic processes. It increases photosynthesis rate and the transfer of photosynthetic products from leaves to reserving organs (Xu et al., 2020). The application of KSB prohibits the destructive effects of drought stress on photosynthesis rate (Chavoshi et al., 2018). However, the NPK treatment, due to the synergistic effects of different GPB, was more effective on photosynthesis rate and kept photosystem II activity under stress conditions (Xie et al., 2018).

Closing stomata and maintaining internal moisture are essential for adapting to the drought conditions. KSB treatment maintained transpiration rate of salt tree under intense drought stress. Potassium plays an essential role in the turgor regulation of guard cells during stomatal movements; potassium deficiency can cause stomata to open and increase transpiration (Asif et al., 2017; Kashtoh and Baek, 2021). Nevertheless, the synergism of different bacteria under NPK treatment was more influential. Also, we found high stomata regulation under drought conditions for plants treated with NPK treatment than control or other biofertilizer treatments. The data obtained from stomatal conductance were consistent with photosynthesis rate and chlorophyll pigments under NPK treatment. This result might be related to the production of abscisic acid around the root media of salt tree seedlings (Yasmin et al., 2017; Arkhipova et al., 2020). Many environmental stresses, including drought stress and salt stress, reduce the electron transfer system, lead to the production of reactive oxygen species, reduce quantum efficiency of photosystem II (Wang et al., 2018) and chlorophyll, and damage to the photosynthetic system (Zhu et al., 2019). We found degradation of chlorophyll pigments at MD and HD level, which were also consistent with the data obtained from photosynthesis measurement. We found that applying KSB and PSB biofertilizers reduced the chloroplast degradation rate, possibly due to the increased nutrient availability of salt tree. A mixture of three biofertilizers (NPK treatment) could effectively increase the electron transfer rate and quantum efficiency of photosystem II by preserving chloroplast pigments and increasing light protection and chlorophyll fluorescence (Khanghahi et al., 2019; ALKahtani et al., 2020).

## 4.3    Biochemical responses of salt tree to drought and biofertilizer

The increase in soluble carbohydrates of slat tree leaves led to the stomata closure and reduced transpiration. Due to the important role of potassium in stomatal regulation and reduction of transpiration rate under stress, KSB treatment, by providing this element to the plant as well as increasing soluble carbohydrates in leaves, reduces transpiration rate and thus maintains the plant water status at a higher level under stress conditions (Jha, 2017; Yasin et al., 2018). Also, the negative correlation between soluble carbohydrates and proline ($r= -0.414$; Table 4) showed negative effects of KSB treatment on proline production.

Proline and phenol osmolytes prevent peroxidation of lipids and cellular structures by

eliminating free radicals and reactive oxygen species, thereby improving the osmotic regulation of the salt tree under stress. Numerous reports have suggested that inoculation of plants with GPB increases proline levels (Chiappero et al., 2019; Ejaz et al., 2020), increases the gene expression of phenolic compounds (Khan et al., 2019b), induces the production of antioxidants, and thus reduces free radicals (Chiappero et al., 2019). The effect of NPK treatment was more profound on phenol content and root dry weight ($r$=0.335; Table 4). This indicate that the synergistic effect of bacteria was more successful than their single application and increased the production of osmolytes, thereby improving physiological traits under stress conditions.

## 5  Conclusions

The growth of arid land plants is mainly limited due to a lack of available nutrients and drought. This study shows that different GPB play different roles in the growth and survival of salt tree in the arid land ecosystems. NFB increases plant growth parameters. Therefore, it enables salt tree to make maximum use of favorable environmental conditions. PSB improves root traits, herby increasing plant tolerance to drought. KSB helps salt tree to regulate photosynthesis rate against drought. However, the synergic effects of three biofertilizers lead to the highest performance of salt tree under all drought and low nutrient treatments. Therefore, unless we are looking to improve specific plant traits, simultaneous application of three biofertilizers would be the most effective treatment for increasing the growth and establishment of salt tree seedlings in degraded lands.

## Acknowledgements

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